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User guide for the

Escherichia coli combined Assay

Array Hybridisation Assay for DNA-based serogenotyping and detection of resistance and virulence genes of *Escherichia coli*

For Research Use Only. Not Intended for Use in Clinical Diagnostics.

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BACKGROUND

The CLONDIAG *Escherichia coli* (*E. coli*) combined Assay allows DNA-based serogenotyping of most known O- and H-antigens as well as, simultaneously, the detection of important anti-microbial resistance (*i.e.* blaOXA) and virulence genes (*i.e* stxA/stxB).

RNA-free, unfragmented genomic DNA from pure and monoclonal *E. coli* colony material is amplified approximately 50-fold and internally labelled with biotin-11-dUTP using a linear amplification protocol. In contrast to standard PCR, only one antisense primer per target is used resulting in producing single stranded (ss) DNA reaction products. This allows simultaneous sequence-specific labelling and amplification of an essentially unlimited number of targets. However, sensitivity is lower than in a standard PCR (whereas contamination with amplicons is nearly impossible) and for that reason the method is restricted to clonal colony material and cannot be performed on samples such as swabs or other patient samples (e.g. faeces). Resulting biotin labelled ssDNA is transferred and hybridised to DNA oligonucleotide microarrays with 393 probes for different genetic markers and a biotin staining control. Probes for serogenotyping are printed once, whereas probes for antimicrobial resistance (AMR) and virulence genes are printed in two spots.

Targets include a variety of species and serotyping markers including genes that code for 23 different O-antigens and 47 H-antigens. Additionally, 102 probes targeting AMR genes as well as 88 probes for virulence genes were included. Spot recognition is performed automatically based on a digital image of the arrays and results are given as an html-file with a description of each analysed target.

GENERAL INSTRUCTIONS FOR USE

Intended Use

For Research Use Only. Not Intended for Use in Clinical Diagnostics.

This assay allows genotypic characterisation of *E. coli* isolates for research and epidemiological applications. It must not be used as a substitute for phenotypic susceptibility tests and for the guidance of antibiotic therapy.

Specifications

Upon receipt, the assay components need to be stored at different temperatures as specified on the package insert. The assay is to be performed at an ambient temperature of 18°C to 28°C.

Technical Support

If you require any further information on this product please contact:

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Safety Precautions

- **The assay is intended for use by personnel that are trained in microbiological and molecular methods. Preparation of DNA from pure *E. coli* colonies (clones) requires expertise in microbiology and the local regulations for handling of pathogenic microorganisms (biosafety level 2) are to be obeyed.**
- **Isolated, cell-free *E. coli* DNA may be processed without further biosafety precautions, although contamination with *E. coli* or other bacteria needs to be ruled out.**
- **Always wear protective clothes as required for laboratory work by your local regulations.**

Material Safety Data Sheets (MSDS)

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)] and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, the enclosed reagents do not require a Material Safety Data Sheet (MSDS). They do not contain more than 1% of a component classified as hazardous and do not contain more than 0.1 % of a component classified as carcinogenic. MSDS therefore are not provided. Nevertheless, the buffers may cause irritation if they come into contact with eyes or skin, and may cause harm if swallowed. The regular precautions associated with laboratory work should be obeyed (e.g., wear protective goggles, gloves and lab coat and avoid contact with the reagents). In case, any liquids are spilled, clean with disinfectant and/or laboratory detergent and water.

Alere assumes no liability for damage resulting from handling or contact with these products. If you have any questions please contact our Technical Support (see above).

Shipping Precautions

RID/ADR: *Kein Gefahrgut* / No dangerous goods

IMDG: No dangerous goods

IATA: No dangerous goods

REAGENTS AND DEVICES

Assay Components, Storage and Stability

All reagents are provided in a certain surplus amount (see below). In case of need, all components may also be ordered separately; please refer to the order numbers at the end of this user guide. For pricing please contact your local representative or our customer service, respectively.

The expiry date can be found on each bottle and on the outer package. All components have been tested for stability for short term shipment (<1 week) at ambient temperature (< 37 °C). The assay components with a rather limited stability are D1 and C3. The other components have proven to be stable even six months after the assay expiry date has passed.

Cell Lysis (optional)

- A1: Lysis Buffer (cat# 245101000)
  Store at 18-28°C (ambient temperature). Surplus: 50%.
- A2: Lysis Enhancer (lyophilised, cat# 245102000)
  Store at 18-28°C (ambient temperature). Centrifuge A2 tubes shortly prior to opening. Add 200 µL Buffer A1 to Lysis Enhancer before use. Mix well and store for less than 1 week at 2-8°C. Sufficient for 96 isolations.

DNA Labelling and Amplification

- $B1^{+}$: Labelling Buffer, Store at 2-8°C. Surplus: 25%.
- B2: Labelling Enzyme, Store at 2-8°C. Surplus: 50%.
- Ecoli-PM1: lyophilized Labelling-Primer-Mix,
  dilute in 50 µl molecular grade water, Store at -20°C,

Hybridisation and Detection

- ArrayStrips (12 x 8 samples),

  Protected against light and sealed under inert gas. Store at 18°C to 28°C. After opening to be used within two weeks. Close the unused wells with caps, protect them against humidity and dust and store them at a dark place. *Avoid any touching or scratching of the surface of the microarray at the bottom of the well. Do not store or handle unused wells at an air humidity of more than 60% since this may irreversibly corrode the spots.*

- CapStrips (24 strips)

- C1: Hybridisation Buffer

  Store at 18-28 °C, protect against sunlight. Surplus: 100%.

- C2: Washing Buffer 1

  Store at 18 °C - 28 °C, protect against direct sunlight. Surplus: 100%.

- C3: HRP Conjugate 100x

  Store at 2-8 °C, protect against direct sunlight. Surplus: 100%.

- C4: Conjugate Buffer

  Store at 18°C to 28°C, protect against direct sunlight. Surplus: 200%.

- C5: Washing Buffer 2

  Store at 18°C to 28°C, protect against direct sunlight. Surplus: 200%.

- D1: Horseradish Peroxidase Substrate

  Store at 2-8°C, protect against direct sunlight. Surplus: 50%.

- CM: Reference DNA from *E. coli* EDL933 (GenBank accession number NC_002655.2),

  c_{DNA} = 0.1-0.4 µg/µL. Store at 2-8 °C. Sufficient for 5-6 tests.

Instrumentation & Software

- ArrayMate Reader (to be ordered separately, for details see below)
  The *E. coli* combined assay may be used on the ArrayMate reader only. The alternative devices ATR01/03 are not suitable for reading ArrayStrip based assays. In case of any questions please contact us.
- Iconoclust software (provided with the reader)
- Test specific software plug-in (can be downloaded from Alere Technologies'/CLONDIAG´s website, check periodically for updates, for details see below). Information (such as spot names, marker names, location of the spots on the array, size of the image taken by the reader's specific camera) is delivered with the reader or can be downloaded from our website. These test-specific plug-ins will occasionally be updated. Please check the NEWS section of our website http://www.alere-technologies.com. Support is available via cct.home@clondiag.com.

Components required but not provided

- Growth media for the cultivation of *E. coli.* The test should be performed with colonies harvested from 2xTY or Columbia Blood Agar. Other rich media (e.g. Standard 1 or LB) may also suffice, but have not systematically been tested. Liquid media should not been used because contaminations or mixed cultures cannot easily be ruled out.
- Equipment and consumables needed for the cultivation of *E. coli* (incubator, inoculation loops, Petri dishes)
- DNA preparation kits:
  The assay has been tested with the DNeasy Blood & Tissue Kit from Qiagen (cat# 69504) and High Pure DNA Isolations Kit from Roche (Cat. No. 11796828001).

  Please note: *The DNA specimen needs to be free of RNA. Recommendation: a pre-treatment with the Cell Lysis components A1/A2 (see below) or a standard RNase A treatment while DNA preparation.*

- Equipment needed for DNA isolation, e.g. pipettes, centrifuge, thermoshaker or automated device (see above)
- Photometer (OD 260 nm) for measuring the concentration of DNA
- Equipment for non denaturing agarose DNA gel electrophoresis for quality control of DNA
- Thermocycler for PCR
- Thermoshaker

  We strongly recommend the BioShake iQ by Quantifoil Instruments (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayStrips. Alternatively, you may use Eppendorf's Thermomixer Comfort, equipped a heating block for microtitre plates.
- Pipettes: suitable for 1µL-5µL volumes, 90µL, 100µL, 200µL, 1000µL
- Multichannel Pipettes for 100-200 µL
- Reaction vials suitable for PCR
- Ultrapure (PCR grade) water
- RNase A (we recommend Qiagen's RNase A solution, 100 mg/mL, Qiagen order# 19101).
- Pasteur pipettes (BRANDT / Cat. No. 612-2856).

PROTOCOL

Culturing and Harvesting Bacterial Cells

Members of the genus *Escherichia* are potential pathogens. All procedures for cultivation of the bacterium and DNA preparation need to be performed by properly trained staff in a biosafety level 2 facility.

Grow *E. coli* on 2xTY or Columbia Blood agar (overnight at 37°C or 48 hrs at room temperature). Obtain confirmation of the identification as *E. coli* (API, VITEK, MALDI) and make sure that you have a pure, monoclonal culture of *E. coli*. Contamination with other bacteria, especially with other *Enterobacteriaceae* needs to be strictly avoided as the might carry the same resistance genes as certain *E. coli* strains and thus can introduce false positive signals and patterns.

Extraction of DNA

The required sample type for the *E. coli* combined assay is 0.5-2 µg (c_{DNA} = 0.1-0.4 µg/µl) of intact genomic DNA from a single clone.

The DNA specimen needs to be free of RNA and it should not be fragmented. This can be determined by agarose gel electrophoresis. Additionally, the microarray includes probes as an internal control for RNA contamination. The automatic software analysis will give a “failed” if a RNA contamination occurred while DNA isolation.

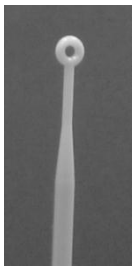
DNA should not be prepared by disrupting *E. coli* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. Most performance problems with the *E. coli* combined assay are due to insufficient amounts or quality of DNA preparation. We therefore strongly recommend following the protocols outlined below.

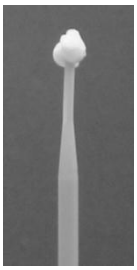
Please note: To yield more genomic DNA, we recommend the optional cell lysis step with A1/A2 reagent.

Extraction of DNA by Spin Columns (e.g. Qiagen)

- Add an inoculating loop full of monoclonal colony material of the *E. coli* isolate to 0.2 ml 1xPBS and vortex thoroughly.

Loop empty Loop full





It is important to harvest enough bacteria; this is a prerequisite for extraction of a sufficient amount of DNA.

Take an inoculating loop of 1mm diameter filled with bacteria as shown in the right picture.

Optional cell lysis with A1/A2 reagent (instead of 1xPBS):

- *Centrifuge A2 tube shortly, open it, add 0.2 ml of Lysis Buffer A1 to Lysis Enhancer A2 and dissolve.*
- *Add an inoculating loop full of monoclonal colony material of the E. coli isolate to this A1/A2 reagent and vortex thoroughly.*
- *Incubate the colony material of the E. coli isolate in A1/A2 for 30-60 min at 37°C and 550 rpm in the thermoshaker.*

- Proceed with the DNA preparation protocol of the DNA preparation kit. For the Qiagen DNeasy Blood&Tissue Kit that is as follows:
- Add 20 µL proteinase K (Qiagen Kit, or equivalent) and add 200 µL buffer AL (Qiagen Kit)
- Vortex shortly or shake vigorously.
- Incubate for 30-60 min at 56°C and 550 rpm in the thermoshaker.
- **important**: if A1/A2 reagent not used, add now 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing
- Add 200 µl ethanol (96-100%)
- Vortex the sample and centrifuge shortly.

- Transfer the complete content of the tube (including any precipitate) into a spin column that is placed in a 2 ml collection tube.
- Centrifuge (8000 rpm, 1 min) at room temperature, time and speed need to be determined depending on viscosity of the sample and type of centrifuge used. All liquid should be collected in the collection tube afterwards.
- Discard collection tube with liquids.
- Place the spin column in a new 2 ml collection tube (provided with the kit).
- Add 500 µl Buffer AW1.
- Centrifuge (8.000 rpm, 1 min) at room temperature.
- Discard collection tube with liquids.
- Place the spin column in a new 2 ml collection tube (provided with the kit).
- Add 500 µl Buffer AW2.
- Centrifuge (14.000 rpm, 3 min) at room temperature, the membrane of the spin column should be dry, and all liquid should be in the collection tube.
- Discard collection tube with liquids.
- Place the spin column in a clean 1.5 ml tube (not provided with the kit).
- Add 100 µl Buffer AE (or PCR grade distilled water) directly onto the membrane of the spin column.
- Incubate at room temperature for 1 min to elute DNA.
- Centrifuge (8000 rpm, 1min) at room temperature.
- Optional: add another 100 µl Buffer AE (or PCR grade distilled water) directly onto the membrane, incubate at room temperature for 1 min and centrifuge again.
- Discard the spin column.

Please note: Ethanol from Washing Buffers strongly inhibits the enzymes used in the assay.

A contamination with Washing Buffer might occur during elution of prepared DNA by drops adhering to the funnel of the spin columns. Thus these funnels should be gently touched and tried with sterile filter paper or wipes prior to the elution step. Alternatively, prepared DNA can shortly be heated to evaporate ethanol (e.g., 10 min at 70 C).

- Check for DNA integrity and absence of RNA (e.g., agarose gel). If necessary, you might perform another digestion step with additional RNase A (not provided). Measure DNA concentration (A_{260} method), it shouldn´t be less than 0.1 µg/µl. The concentration might be increased by heating and evaporation of water, or by using a speed vac centrifuge.

Linear Amplification and Internal Biotin Labelling

Please keep in mind the limited surplus of reagents whilst pipetting. The surplus of $B1^+$ labelling reagent is 25 %.

- Prepare a Master Mix by combining 3.9 µL of $B1^+$ labelling reagent, 1 µl Ecoli-PM1 Labelling-Primer-Mix and 0.1 µL of B2 (DNA polymerase) per sample.
- Add 5 µl of *E. coli* DNA (c_{DNA} = 0.1 - 0.4 µg/µl) prepared as described above to 5 µl of the Master Mix ($B1^+$/B2). Do not forget to label the vial!
- Perform amplification in a pre-programmed thermocycler (e.g., Eppendorf Mastercycler gradient with heated lid, VWR, cat No. 460-0108) according to following protocol:

Pre-heat cover/lid to 105°C	
300 sec at 96°C	
50 cycles with	20 sec at 50°C
	40 sec at 72°C
	60 sec at 96°C
Cool down to 4°C, hold	

- the samples can be stored frozen until usage

Please note: When using another device, some adaptations might be necessary. Before starting routine use, please test the protocol with a few known reference strains.

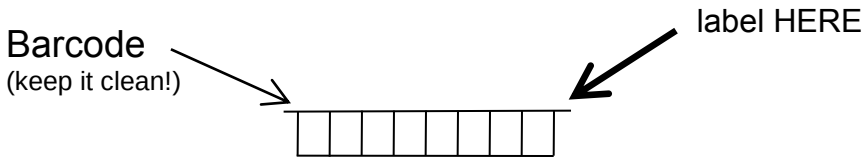
Hybridisation

General Remarks - Handling of Arrays

- *Never touch the array surface!*
- *Avoid complete drying of the array surface during processing!*
- *Do not allow it to stay without liquid for more than two minutes!*
- *Never rinse the wells with distilled water after the hybridisation step, use only C2 Washing Buffer!*

Unused wells should be capped during the whole procedure. The strips may be processed up to three times without a loss of quality of properly capped unused arrays. Close all wells that will not be used with a cap und leave it there until you use these wells (for storage conditions after use: see section “Assay components, storage and stability/Hybridisation and Detection”).

Always label your array strips with a laboratory marker at the recommended position. Never label them on the bottom or across the data matrix barcode! This may cause an error.



Avoid contact of data matrix barcode with organic solvents! The ArrayMate needs the information encoded in the data matrix to perform the assay and the analysis afterwards.

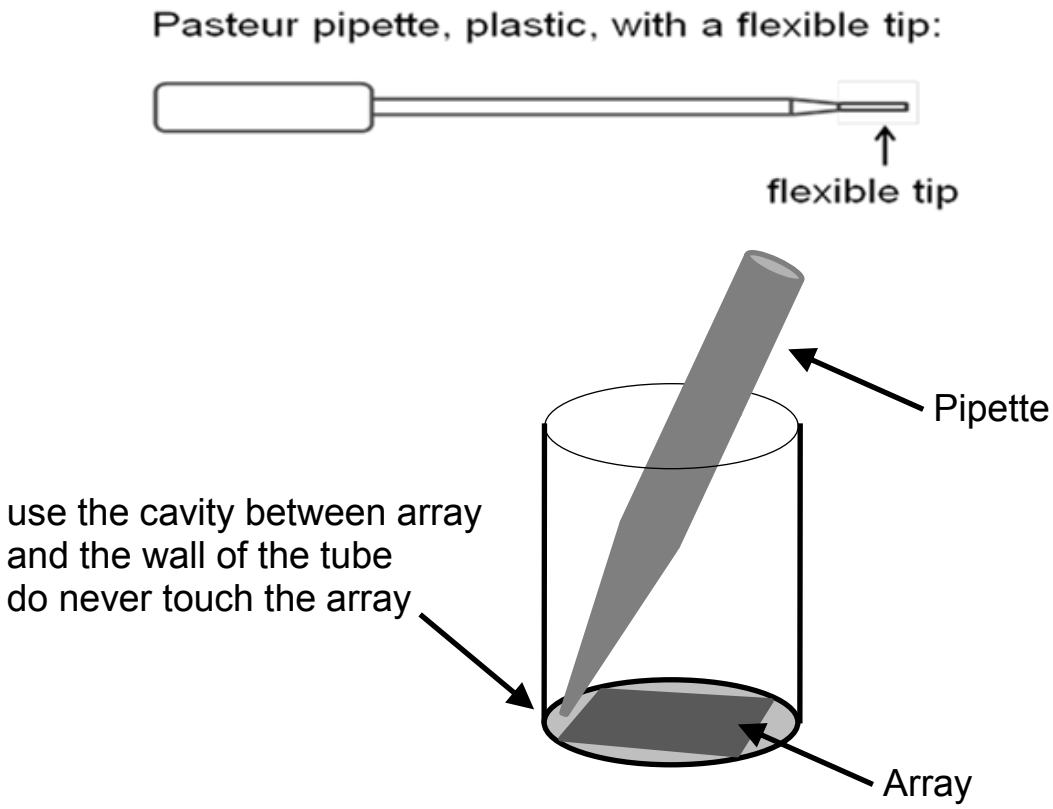
Avoid touching the bottom of the microarray strip and keep it clean.

General Remarks - Handling of Liquids

We recommend the use of a multichannel pipette and reagent reservoirs. Please keep in mind the limited surplus of C1 (100%).

We strongly recommend that the liquid is removed by pipetting rather than by inverting the strips and flicking the liquids out. Fine tipped soft, disposable Pasteur pipettes are suited best (such as BRANDT / Cat. No. 612-2856). Always place the pipette tip at the cavity between the

array and the wall of the reagent well. If you touch the array surface, probes may be scratched off and this may cause an error.



General Remarks – the Substrate (Precipitating Dye) D1

An appropriate amount of substrate (precipitating dye) should be filled into an Eppendorf tube and taken out of the refrigerator when starting the procedure allowing it to pre-warm to room temperature/25°C. Cold D1 may yield weak signals. D1 should shortly be centrifuged prior to use to remove bubbles as well as possible precipitates.

Triggered by peroxidase, in case of positive reactions, the dye precipitates but it is not covalently bound. The precipitate can be dissolved by vigorous shaking. Thus the arrays must not be shaken, dropped or moved abruptly during the staining procedure and afterwards.

After completion of staining, remove and discard reagent D1 as completely as possible and scan immediately. The dye precipitate fades slowly in presence of liquids.

General Remarks - Thermoshakers

The correct temperature within the vessels is essential; therefore always use the appropriate equipment for heating. Because of a possibly inhomogeneous distribution of the temperature within the heating block and because of possible differences between displayed and actual temperatures, the use of different brands of thermoshakers might affect test performance. We tested the assay with BioShake iQ by Quantifoil Instruments (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayStrips and Eppendorf's Thermomixer Comfort, equipped with a heating block for microtitre plates.When using other devices, some modifications to the protocol might be necessary. Before starting routine use, please test the protocol with a few known reference strains or the control DNA (*E. coli* EDL933).

Protocol for Quantifoil's BioShake iQ

- Switch on the thermoshaker and let it pre-heat to 50°C.
- Remove the ArrayStrip(s) from the pouch.
- Insert the ArrayStrip(s) into the white frame. Assure the correct orientation (data matrix barcode close to row (A) and proper fit.
- Pre-wash the array in two steps:
- First, PCR-grade distilled water, 200 µl per well at 50°C, 5 min and 550 rpm
- Second, C1 Hybridisation Buffer, 150 µl per well at 50°C, 5 min and 550 rpm
- Add 90 µL of buffer C1 to each tube with (10 µL) labelled amplification product, mix gently
- Remove the buffer from the well with the array and add the mixture of C1 and labelled amplification product
- Incubate at 50°C, 60 min and 550 rpm.

- Remove liquid and add 200 µl C2 Washing Buffer. Incubate at 45°C, 10 min and 550 rpm, remove and discard.
- Add another 200 µl C2 Washing Buffer. Incubate at 45°C, 10 min and 550 rpm, remove and discard.
- Meanwhile, prepare conjugate: for each experiment add 1 µl conjugate 100xHRP to 100 µl C4 Conjugation Buffer. This mixture is stable for around one working day at room temperature; C3 is delivered with a surplus of 100%, C4 is delivered with a surplus of 200%.

  Suggested pipetting scheme:

	1 well	2-3 wells	4-6 wells	7-10 wells	11-15 wells	16-20 wells	21-30 wells	31-40 wells
C3	1.5 µL	3.5 µL	7 µL	11 µL	16 µL	21 µL	32 µL	42 µL
C4	150 µL	350 µL	700 µL	1100 µL	1600 µL	2100 µL	3200 µL	4200 µL

- Remove and discard Washing Buffer, and add 100 µl diluted conjugate to each well, incubate at 30 °C, 10min and 550rpm.
- Add 200 µl C5 Washing Buffer. Incubate at 30°C, 5 min and 550rpm.
- Remove and discard Washing Buffer, add 100 µl of D1 substrate (precipitating dye, at 25°C, see above) per well.
- Incubate at 25°C, 10 min *but* ***do not shake !***
- Remove liquid completely.
- The bottom of the ArrayStrips may be cautiously be cleaned with wipes, bubbles may be removed by removing and adding D1.
- Scan and process (see below).

Protocol for Eppendorf's Thermomixer Comfort with microtitre plate adapter

- Switch on the thermoshaker and let it pre-heat to 55°C.
- Remove the ArrayStrip(s) from the pouch.

- Insert the ArrayStrip(s) into the white frame. Assure the correct orientation (data matrix barcode close to row (A) and proper fit.
- Pre-wash the array in two steps:
- First, PCR-grade distilled water, 200 µl per well, just pipette up and down four times, remove and discard.
- Second, C1 Hybridisation Buffer, 150 µl per well at 55°C, 5 min and 550 rpm
- Add 90 µL of buffer C1 to each tube with (10 µL) labelled amplification product, mix gently
- Remove the buffer from the well with the array and add the mixture of C1 and labelled amplification product
- Incubate at 55°C, 60 min and 550 rpm.
- Remove liquid and add 200 µl C2 Washing Buffer. Incubate at 40°C, 12 min and 550 rpm, remove and discard.
- Add another 200 µl C2 Washing Buffer. Incubate at 40°C, 12 min and 550 rpm, remove and discard.
- Meanwhile, prepare conjugate: for each experiment add 1 µl conjugate 100xHRP to 100 µl C4 Conjugation Buffer. This mixture is stable for around one working day at room temperature; C3 is delivered with a surplus of 100%, C4 is delivered with a surplus of 200%.

  Suggested pipetting scheme:

	1 well	2-3 wells	4-6 wells	7-10 wells	11-15 wells	16-20 wells	21-30 wells	31-40 wells
C3	1.5 µL	3.5 µL	7 µL	11 µL	16 µL	21 µL	32 µL	42 µL
C4	150 µL	350 µL	700 µL	1100 µL	1600 µL	2100 µL	3200 µL	4200 µL

- Remove and discard Washing Buffer, and add 100 µl diluted conjugate to each well, incubate at 30°C, 10min and 550rpm.
- Remove liquid and wash with 200 µl C5 Washing Buffer, just pipette up and down four times, remove and discard.

- Add another 200 µl C5 Washing Buffer, just pipette up and down four times.
- Remove and discard Washing Buffer, add 100 µl of D1 substrate (precipitating dye, at 25°C, see above) per well.
- Incubate at 25°C, 10 min *but* ***do not shake !***
- Remove liquid completely.
- The bottom of the ArrayStrips may be cautiously be cleaned with wipes, bubbles may be removed by removing and adding D1.
- Scan and process (see below).

Data Analysis

Starting the ArrayMate Reader

We recommend starting the ArrayMate Reader after having started the hybridisation; this allows you to conveniently start the device and to import the worklist file (see below)

Please note that this is a short instruction only. For more detailed information please refer to the ArrayMate User Manual.

- Switch on the ArrayMate (main switch on the rear below the electric cable plug, operating switch on the bottom/left corner of the front side).
- Switch on the screen (switch right hand below the screen).
- Log-in as "R&D User" (Research and Development User) for full access to test specific software (a default password will be provided together with the ArrayMate device). If you log-in as "User", you will obtain only raw values, but no interpretation as positives/negatives and no strain assignment. "Administrator" log-in will allow manipulation of file folders and software; and this should be done only upon direct advice of Clondiag´s IT team.

- The user interface will be loaded, ArrayMate performs internal testing. This will require slightly less than a minute.
- Click on the icon “New Run” (left upper edge of the screen). A suggestion for a run name / folder name for the new run appears in the top line of the screen). You may modify or change the experiment name at your convenience.
- Type in your operator ID (**optional**).
- You may optionally enter a comment into the “memo” field.

Worklist

A “Worklist” file allows linking an identifier such as a laboratory/sample number to a position of an array within the ArrayStrip. For privacy reasons, arrays should not be identified by patient names. Worklists can be generated using spreadsheet software such as EXCEL (see below) but must be saved in the *.txt file format that can be imported into the test specific ArrayMate software. Do not use special characters (such as : ; ()[] / \ ä ü etc.).

- Create a list with at least three columns that have headers written into the first line. The following headers are obligatory (in this order): position / sample ID / assay ID (Table 1).
- Positions are continuously numbered from 1 to, maximal, 96. Position 1 would correspond to A1, 8 to H1, 9 to A2 and 96 to H12 (Table 2). Do not leave empty lines in the worklist. If you use EXCEL, position numbers should be typed into column A.
- Sample ID are strain/sample/laboratory numbers such as exported from your LIMS (or assigned in any different way). Patient´s names should not be used as Sample IDs.
- Assay IDs allow the system to identify the actual test and to correctly use information on layout, spot number and identity etc. *E. coli combined* Assay has the Assay ID: 10313. *Assay ID numbers must not be confused as this could lead to errors or loss of data.*
- You may add further columns and headers with notes and comments at your convenience Information from these columns will not appear on the result screens or the Test Report.
- We recommend using a printout of the worklist as template for pipetting.

- Safe the worklist as tab separated *.txt file on the memory stick provided together with the ArrayMate.
- To avoid confusion, make sure that worklists are named unambiguously or that worklists from earlier experiments are deleted.

Table 1: Example worklist:

Position	sampleID	assayID	comment
1	2013-12345	10313	
2	2013-12346	10313	
3	2013-12347	10313	
4	2013-12348	10313	
5	2013-12349	10313	
6	2013-12350	10313	
7	987654	10313	*Isolate referred from Dr. J. Doe.*
8	*E. coli* EDL933	10313	*Control strain*

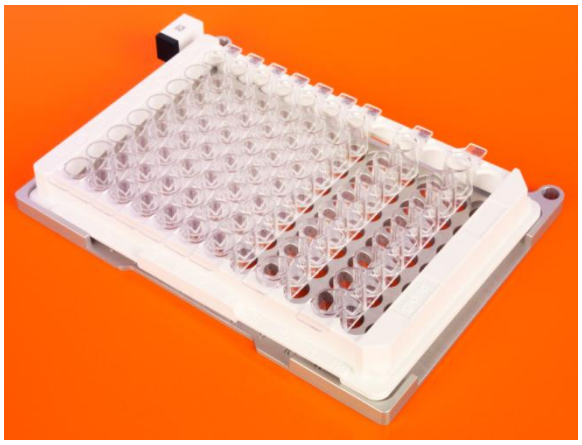
Table 2: Positions in the 96 well format

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
H	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Data Acquisition in the ArrayMate Reader

- Insert your memory stick containing the worklist. Use any of the USB ports down to the right side of the ArrayMate.
- Press the button: [...]; a folder selection dialog will open.
- Select your worklist (path: “My Computer/Removable Disk”).

- Open your selected worklist with “Enter” or the button “Open”.
- Press the button: (your imported worklist opens in a separate window). Proofread. If the new window is empty or if it was the wrong worklist, repeat the import.
- Press the button “OK”; the worklist window will close.
- Leave the memory stick attached to the ArrayMate if you intend to export *E. coli combined* Test Reports afterwards.
- Press the button “next” (bottom/right on the screen; reader is opening).
- Carefully insert the appropriate metallic adapter/frame into the ArrayMate. Do not apply any strong force. Assure proper fit, otherwise the images may be out of focus.
- Carefully insert the white frame with the array strips into the metallic adapter. Assure the correct orientation (Position A1 in the frame next to the data matrix barcode on the adapter) and proper fit, otherwise the images may be out of focus.



ArrayStrip frame with inserted strips. Strips are inserted in accordance to the worklist.

Please note: *ArrayStrips must be clean. They should not contain any liquids by now. Barcodes must be clean. There must be no lids on the wells that are to be analysed (however, unused wells should remain capped).*

- Press the button “Next” (bottom/right on the screen; reader is closing, analysis program starts, it takes ca. 2-10 min dependent on the number of strips; reader takes images and automatically analyses the data). The progress of the reading is indicated by the following symbols:

photographed: in analysis: ready:

- The reader indicates the end of the entire process with an acoustic signal (beep).
- Press the button “Next” (bottom/right on the screen; reader is opening).
- Remove the white frame with the ArrayStrip(s).
- Press the button “Next” (bottom/right on the screen; reader is closing).

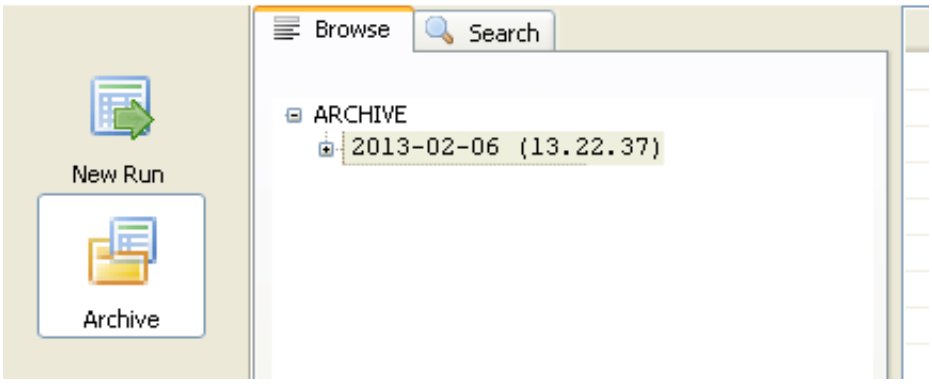
Results

On the left hand site of the screen you will see a list showing all runs stored on the ArrayMate´s hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not visible:

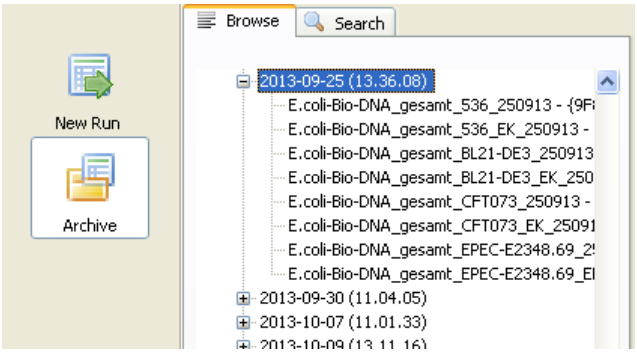
- Press the button “Archive” (left hand) and activate the Flag “Browse” (top left).

The runs are organised like folders in “Windows Explorer” and, by default, named according to the date of acquisition.

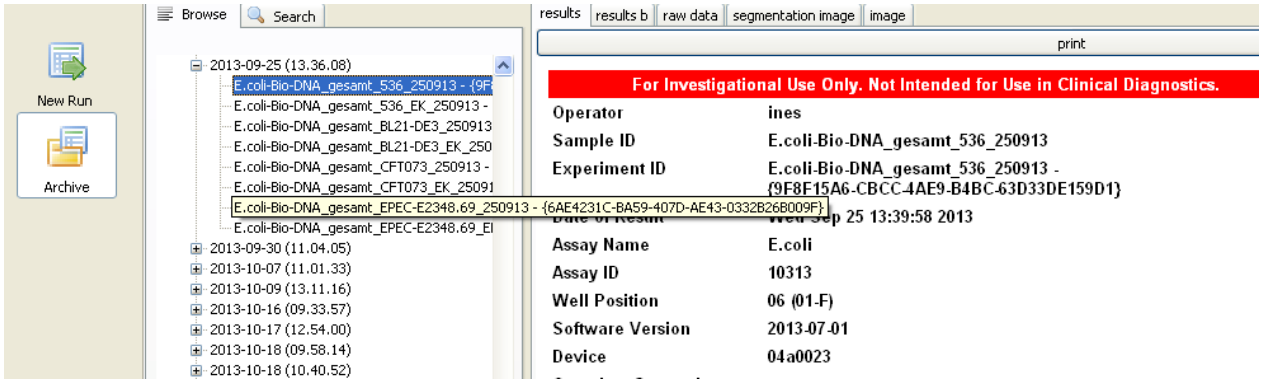
Example: there is one reading in this archive:



If you click on the plus symbol left on the run name, the folder opens and you will see a list of the individual arrays alphabetically ordered by Sample ID.



Click on a Sample ID and the *E. coli combined* Test Report for this array is shown in the window on the right:



Export of *E. coli combined* Test Reports

The generated result files in an html format will show information of all target genes. Possible invalid controls that might display in this report will be explained below (see Troubleshooting).

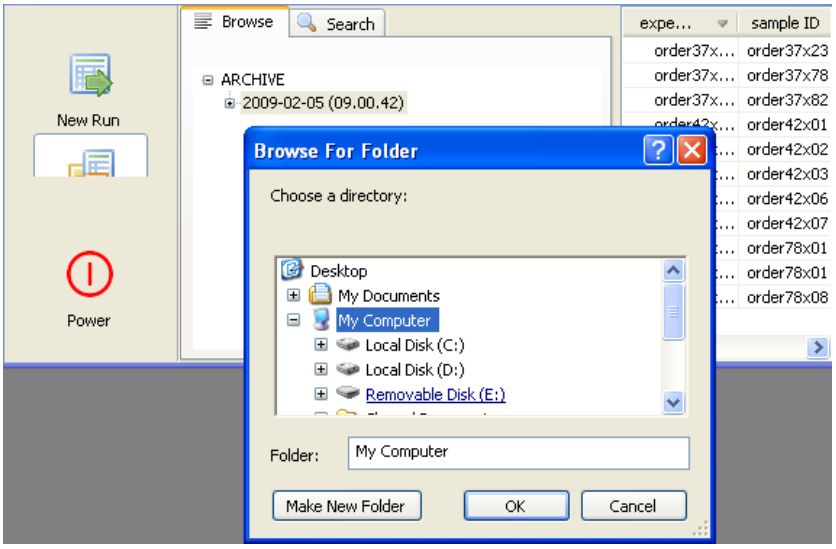
Other files that are generated and that can be exported include

- a *.txt file with the raw measurements,
- an image file (*.bmp) with the actual photo of the array,

- a second image file (*.png) in which the coordinate grid is superimposed and the recognised spots are circled and
- a *.xml files that contains the same information as the html result sheets for future export into databases etc.

Please note: only complete runs can be exported. The export of individual E. coli combined Test Reports is not possible

- Right-Click on the reading (a menu appears with the option “Export Run Reports”).
- Right-Click on “Export Run Reports” (a file browser opens).



- Click on “My Computer”, then on “Removable Disk” and choose the folder where to store or click on the button “Make New Folder” (on the bottom; a new folder icon appears).
- Rename the new folder (e.g. with the experiment name or date).
- Click on the “Ok” button (data are exported now into the new folder on your memory stick).
- Do NOT remove the memory stick as long as the hourglass symbol is visible.
- Switch off the device by clicking on the “Power”-button (left/down on the screen):
- Switch off the Screen. There is no need to physically switch off the ArrayMate.

TROUBLESHOOTING

In case of trouble always make sure that reagents are within the recommended shelf-life and stored in the appropriate way.

In case of trouble we are always happy to support. Please contact, cct.home@clondiag.com and please include a description of the problem as well as the array images (*.bmp files) in question.

Staining Control

A staining control is included to check whether possible problems originate from the hybridisation or the staining procedure. If the staining control has “Failed” proceed as follows:

Horseradish peroxidase conjugate may have degraded during storage. Add 1µL buffer C3/C4 to 9 µL D1 (substrate). If the solution turns green within 3-5 seconds, the horseradish peroxidase still has sufficient enzymatic activity.

Enzymatic reaction is inhibited by carryover of buffer C1. Ensure proper washing of the wells to remove all of Buffer C1 prior to adding horseradish peroxidase conjugate.

If the Staining Control has “Passed”, refer to the following hints.

Image Quality

In case of poor image quality we recommend to re-check DNA quantity and quality first by loading leftover DNA on an agarose gel.

In order to determine whether any problems originated from the DNA preparation, perform an experiment with the CM / Control material. This is DNA from the reference strain E. coli EDL933 (GenBank accession number NC_002655.2) and should be identified by the assay as *E. coli* with O:146 and H:20. If the control experiment yields a valid result and a correct identification, there was probably an issue with DNA preparation. If the control experiment also fails, an error affecting later steps or a degradation of reagents from later steps is likely.

DNA Quality and RNA contamination control

The amount of DNA is crucial because of the linear kinetics of amplification (see Introduction). DNA should be free of RNA, as free RNA reduces the efficiency of amplification and labelling by effectively removing primer from the reaction mix due to competitive hybridisation. A_{260} readings will cover RNA and other contaminants as well. Therefore pure DNA preparations without RNA contaminations are a prerequisite for proper DNA concentration measurement. RNase treatment prior to A_{260} reading therefore is necessary (component A2 contains RNAse). Additionally, the microarray includes probes as an internal control for RNA contamination. The automatic software analysis will give a "failed" if a RNA contamination occurred while DNA isolation.

DNA must be unfragmented, as fragmentation reduces the efficiency of amplification and labelling due to the distance between primer and probe binding sites. DNA should for this reason not be prepared by disrupting *E. coli* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. We made good experiences with the manual QIAGEN DNeasy kit and the Roche High Pure Kit.

DNA must be free of any traces of ethanol, as ethanol strongly influences the amplification. It is possible to heat the sample prior to adding it to the labelling mix (5-10 minutes at 70°C).

Physical Damage to the Array

Scratching of the array surface with a pipette tip can lead to the damage of array spots that prohibits the acquisition of a valid signal. In this case the respective marker is not assigned as "negative", but instead the message "none" appears next to the marker name.

Report Unavailable

If the ArrayMate indicates that no report is available for an array (or multiple arrays on one strip), please check that the strip was positioned properly into the frame. Scratches or drops of condensed water might render the barcode identifier unreadable, please wipe it carefully or try to manually identify the test. If no obvious reason for the fault can be discovered, please contact the technical service.

ADDITIONAL INFORMATION

Warranty

Alere guarantees the performance as described in this user guide. Usage of the Assay was successfully tested at ambient temperatures up to 37°C, a guarantee is limited to ambient temperatures in the laboratory between 18°C and 28°C. Assay components comprise the arrays and their caps, the Lysis Enhancer, the reagents for DNA labelling and for detection of labelled DNA products on the array, the ArrayMate reader and its software. In case one of these components fails within the expiry date due to other reason than misuse, contact Alere for replacement or refund. Terms and conditions apply.

If you have any problem or question, please contact the technical service.

Disclaimer

This system is for research use only.

We do not accept any liability for damages caused by misuse. Misuse comprises, especially but not exclusively, of a use of the system for the detection of resistance genes in order to predict phenotypic antibiotic resistances or susceptibilities for the guidance of an antibiotic chemotherapy.

Since resistances might be caused by genes or mutations not covered by this array or by hitherto unknown genes or mutations, any antibiotic chemotherapy MUST be guided by phenotypic susceptibility tests.

Furthermore, we do not accept any liability for damages caused by inappropriate use of the device as a personal computer, for instance related to the use of additional software, to network connections, or to a breach of privacy related to the storage of confidential information (such as names of patients from whom *E. coli* was isolated) on its hard disk and/or to the use of external storage devices that might be contaminated with spyware.

Quality Control

Each batch is stringently tested with the use of standard *E. coli* DNA preparations for good performance and correctness of results.

List of Components for Separate Order

If required, these reagents for the *E. coli combined* Assay may be ordered separately:

component	name	Category	amount	cat#	storage
A1	Lysis Buffer	Buffer	30 ml	245101000	18-28 °C
A2	Lysis Enhancer	lyophylised enzymes	96 units	245102000	18-28 °C
Ecoli-PM1	PrimerMix *E. coli*_combined (= *E. coli*_gesamt)	Labelling Primer	50 µl/tube	on request	2-8 °C
$B1^+$	Labelling Buffer	buffered reagents	700 µl	245103000	2-8 °C
B2	Labelling Enzyme	buffered enzyme	20 µl	245104000	2-8 °C
C1	Hybridisation Buffer	buffered reagents	30 ml	245105000	18-28 °C
C2	Washing Buffer 1	buffer	120 ml	245106000	18-28 °C
C3	HRP Conjugate 100x	buffered enzyme	200 µl	245107000	2-8 °C
C4	Conjugate Buffer	buffered reagents	30 ml	245108000	18-28 °C
C5	Washing Buffer 2	buffer	120 ml	245109000	18-28 °C
D1	HRP Substrate	buffered reagents	15 ml	245110000	2-8 °C
CM	Control Material	E. coli EDL933 DNA (c_{DNA} = 0.1-0.4 µg/µl)	30 µl	on request	2-8 °C
ArrayStrips	E.coli-combined-AS-2 (=E.coli-gesamt-AS-2)	plugged microarrays	12 St	240008929	15-28 °C
StripCaps	StripCaps	plasticware	24 St	245112000	15-28 °C

For pricing please contact us:

Legal Manufacturer

Alere Technologies GmbH

Loebstedter Str. 103-105

07749 Jena, Germany

Contact

If you require any further information on this product please contact: cct.home@clondiag.com

LITERATURE

Anjum MF, Mafura M, Slickers P, Ballmer K, Kuhnert P, et al. (2007) Pathotyping *Escherichia coli* by using miniaturized DNA microarrays. Appl Environ Microbiol 73: 5692-5697.

Ballmer K, Korczak BM, Kuhnert P, Slickers P, Ehricht R, et al. (2007) Fast DNA serotyping of *Escherichia coli* by use of an oligonucleotide microarray. J Clin Microbiol 45: 370-379.

Geue L, Schares S, Mintel B, Conraths FJ, Muller E, et al. (2010) Rapid microarray-based genotyping of enterohemorrhagic *Escherichia coli* serotype O156:H25/H-/Hnt isolates from cattle and clonal relationship analysis. Appl Environ Microbiol 76: 5510-5519.

Korczak B, Frey J, Schrenzel J, Pluschke G, Pfister R, et al. (2005) Use of diagnostic microarrays for determination of virulence gene patterns of *Escherichia coli* K1, a major cause of neonatal meningitis. J Clin Microbiol 43: 1024-1031.

Monecke S, Mariani-Kurkdjian P, Bingen E, Weill FX, Baliere C, et al. (2011) Presence of enterohemorrhagic *Escherichia coli* ST678/O104:H4 in France prior to 2011. Appl Environ Microbiol 77: 8784-8786.

Schilling AK, Hotzel H, Methner U, Sprague LD, Schmoock G, et al. (2012) Zoonotic agents in small ruminants kept on city farms in southern Germany. Appl Environ Microbiol 78: 3785-3793.

Wu G, Ehricht R, Mafura M, Stokes M, Smith N, et al. (2012) *Escherichia coli* isolates from extraintestinal organs of livestock animals harbour diverse virulence genes and belong to multiple genetic lineages. Vet Microbiol 160: 197-206.

UPDATES & SOFTWARE

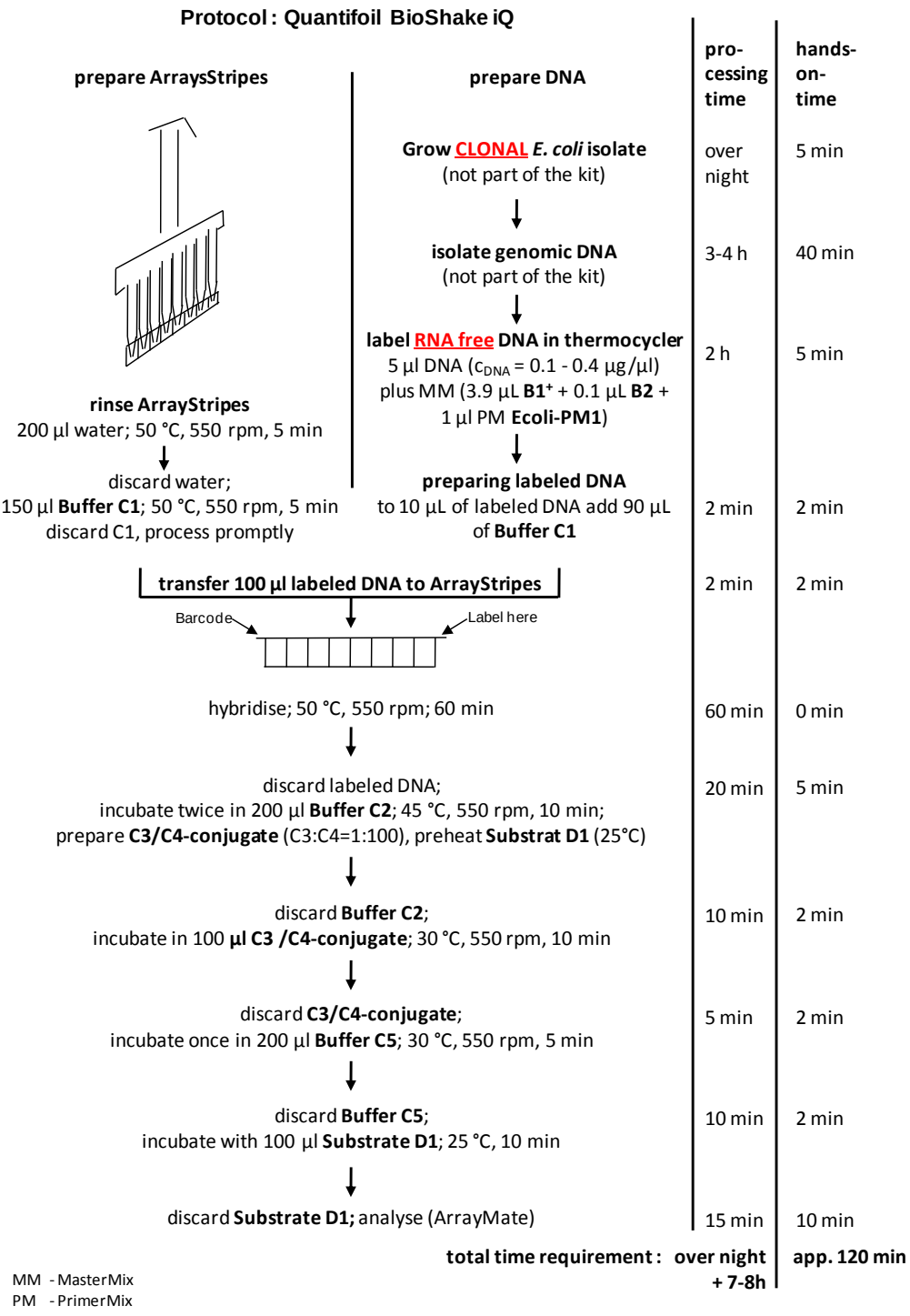
Notifications on database/software updates and freeware tools can be found at :

http://alere-technologies.com/en/science-technologies/publications/downloads.html.

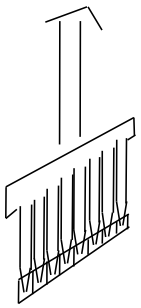
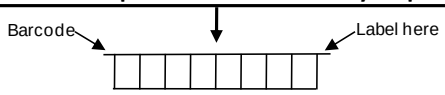
and/or http://alere-technologies.com/en/news.html.

APPENDIX 1 – Flow charts

The figures summarise the test procedure. However, please refer to the text section of this user guide at any step of the test protocol for further important details.


Protocol : Quantifoil BioShake iQ
prepare ArraysStripes
prepare DNA
pro-cessing time
hands-on-time
Grow CLONAL E. coli isolate
(not part of the kit)
over night
5 min
isolate genomic DNA
(not part of the kit)
3-4 h
40 min
label RNA free DNA in thermocycler
5 µl DNA (cDNA = 0.1 - 0.4 µg/µl)
plus MM (3.9 µL B1+ + 0.1 µL B2 +
1 µl PM Ecoli-PM1)
2 h
5 min
rinse ArrayStripes
200 µl water; 50 °C, 550 rpm, 5 min
discard water;
150 µl Buffer C1; 50 °C, 550 rpm, 5 min
discard C1, process promptly
preparing labeled DNA
to 10 µL of labeled DNA add 90 µL
of Buffer C1
2 min
2 min
transfer 100 µl labeled DNA to ArrayStripes
Barcode
Label here
2 min
2 min
hybridise; 50 °C, 550 rpm; 60 min
60 min
0 min
discard labeled DNA;
incubate twice in 200 µl Buffer C2; 45 °C, 550 rpm, 10 min;
prepare C3/C4-conjugate (C3:C4=1:100), preheat Substrat D1 (25°C)
20 min
5 min
discard Buffer C2;
incubate in 100 µl C3 /C4-conjugate; 30 °C, 550 rpm, 10 min
10 min
2 min
discard C3/C4-conjugate;
incubate once in 200 µl Buffer C5; 30 °C, 550 rpm, 5 min
5 min
2 min
discard Buffer C5;
incubate with 100 µl Substrate D1; 25 °C, 10 min
10 min
2 min
discard Substrate D1; analyse (ArrayMate)
15 min
10 min
total time requirement :
over night + 7-8h
app. 120 min
MM - MasterMix
PM - PrimerMix

Protocol : Eppendorf Thermoshaker[a)]

prepare ArraysStripes	prepare DNA	pro-cessing time	hands-on-time
	Grow CLONAL *E. coli* isolate (not part of the kit)	over night	5 min
	↓		
	isolate genomic DNA (not part of the kit)	3-4 h	40 min
	↓		
	label RNA free DNA in thermocycler 5 µl DNA (c_{DNA} = 0.1 - 0.4 µg/µl) plus MM (3.9 µL **B1**$^{+}$ + 0.1 µL **B2** + 1 µl PM **Ecoli-PM1**)	2 h	5 min
	↓		
	preparing labeled DNA to 10 µL of labeled DNA add 90 µL of **Buffer C1**	10 min	5 min
 **rinse ArrayStripes** 200 µl water; pipette up and down (4x)			
↓			
discard water; 150 µl **Buffer C1**; 55 °C, 550 rpm, 5 min discard C1, process promptly			
transfer 100 µl labeled DNA to ArrayStripes		2 min	2 min
			
hybridise; 55 °C, 550 rpm; 60 min		60 min	0 min
↓			
discard labeled DNA; incubate twice in 200 µl **Buffer C2**; 40 °C, 550 rpm, 12 min; prepare **C3/C4-conjugate** (C3:C4=1:100), preheat **Substrat D1** (25°C)		25 min	5 min
↓			
discard **Buffer C2**; incubate in 100 **µl C3 /C4-conjugate**; 30 °C, 550 rpm, 10 min		10 min	2 min
↓			
discard **C3/C4-conjugate**; incubate once in 200 µl **Buffer C5**; 30 °C, 550 rpm, 5 min		5 min	2 min
↓			
discard **Buffer C5**; incubate with 100 µl **Substrate D1**; 25 °C, 10 min		10 min	1 min
↓			
discard **Substrate D1;** analyse (ArrayMate)		15 min	10 min
	total time requirement :	**over night + 7-8h**	**app. 120 min**

MM - MasterMix
PM - PrimerMix

[a)] with heating block for microtitre plates

APPENDIX 2 – PROBE TO TARGET TABLE

Target genes	Probes	Function
Family and Species Marker		
gapA	prob_gapA_611	glyceraldehyde 3-phosphate dehydrogenase A (CP000468.1, locus tag APECO1_847)- genetic marker for family Enterobacteriaceae
ihfA	prob_ihfA_611	integration host factor subunit alpha (U00096.2)- genetic marker for family Enterobacteriaceae
gad	gad_10	glutamate decarboxylase (AE014075.1, locus tag c4328) - genetic marker for genus Escherichia/Shigella
dnaE	hp_dnaE_612, hp_dnaE_613	DNA polymerase III subunit alpha - genetic marker for species Escherichia spec. and Shigella spec. (U00096.2)
rrs	hp_rrs_611, hp_rrs_612	16S rRNA - genetic marker for species E. coli (U00096.2)
O-serotyping		
O4	wzy-O4_11	O-antigen - O4 (U39042.1)
O6	wzx-O6_11, wzy-O6_11	O-antigen - O6 (AJ426045.2)
O7	wzx-O7_11, wzy-O7_11	O-antigen - O7 (AB490074.1)
O8	wzx-O8_11, wzy-O8_11	O-antigen - O8 (AF013583.1)
O9	wzx-O9_11, wzy-O9_11	O-antigen - O9 (wbdA_O9: D43637.1, wzx_O9: AF104912.2, wzy_09: AB031867.1)
O15	wzx-O15_11, wzy-O15_11	O-antigen - O15 (CP002291.1)
O26	wzx-O26_11, wzy-O26_11	O-antigen - O26 (AF529080.1)
O52	wzm-O52_11	O-antigen - O52 (AY528413.1)
O53	wzy-O53_11	O-antigen - O53 (AF402312.1)
O55	wzx-O55_11, wzy-O55_11	O-antigen - O55 (AF461121.1)
O79	wbdU-O79_11, wzx-O79_11	O-antigen - O79 (EU294162.1)
O86	wzx-O86_11, wzy-O86_11	O-antigen - O86 (AY670704.1)
O91	wzx-O91_11, wzy-O91_11	O-antigen - O91 (AY035396.1)
O101	wz-O101_11, wbdA-O9_11	O-antigen - O101 (X59852.1)
O103	wzx-O103_11, wzy-O103_11	O-antigen - O103 (AY532664.1)
O104	wzx-O104_11, wzy-O104_11	O-antigen - O104 (AF361371.1)
O111	wbdH-O111_11, wbdM-O111_11, wzx-O111_11, wzy-O111_11	O-antigen - O111 (AF078736.1)
O113	wzx-O113_11, wzy-O113_11	O-antigen - O113 (AF172324.1)
O114	wzx-O114_11, wzy-O114_11	O-antigen - O114 (AY573377.1)
O121	wzx-O121_11, wzy-O121_11	O-antigen - O121 (AY208937.1)
O128	wzx-O128_11, wzy-O128_11	O-antigen - O128 (AY217096.1)
O157	rfbE-O157_11, wzx-O157_11	O-antigen - O157 (AB008676.1)
O172	wzx-O172_11, wzy-O172_11	O-antigen - O172 (AY545992.1)

H-serotyping		
fliC H01	fliC-H01_11, fliC-H01_12	flagellin C H-antigen H01 (AE014075.1)
fliC H02	fliC-H02_11	flagellin C H-antigen H02 (AF543692.1)
flkA H03	flkA-H03_11	flagellin A H-antigen H03 (AB128916.1) (Note: If repressor FljA "positive", the gene fliC H16 is repressed; H-phenotype is H03)
fliC H04	fliC-H04_11	flagellin C H-antigen H04 (AY249989.1)
fliC H05	fliC-H05_11	flagellin C H-antigen H05 (AY249990.1)
fliC H06	fliC-H06_11	flagellin C H-antigen H06 (AY249991.1)
fliC H07	fliC-H07_11, fliC-H07_12	flagellin C H-antigen H07 (AB028474.1)
fliC H08	fliC-H08_11	flagellin C H-antigen H08 (AJ884571.1)
fliC H09	fliC-H09_11	flagellin C H-antigen H09 (AY249994.1)
fliC H10	fliC-H10_11	flagellin C H-antigen H10 (AY249995.1)
fliC H11	fliC-H11_11	flagellin C H-antigen H11 (AY337465.1)
fliC H12	fliC-H12_11	flagellin C H-antigen H12 (AY249997.1)
fliC H14	fliC-H14_11	flagellin C H-antigen H14 (AY249998.1)
fliC H15	fliC-H15_11	flagellin C H-antigen H15 (AY249999.1)
fliC H16	fliC-H16_11	flagellin C H-antigen H16 (AB128919.1), Note: If repressor fljA "positive", fliC H16 might be repressed, H-phenotype is than determined by flkA H03.
fliC H18	fliC-H18_11	flagellin C H-antigen H18 (AY250001.1)
fliC H19	fliC-H19_11	flagellin C H-antigen H19 (AY250002.1)
fliC H20	fliC-H20_11	flagellin C H-antigen H20 (AY250003.1)
fliC H21	fliC-H21_11	flagellin C H-antigen H21 (DQ862122.1), Note: If repressor fljA "positive", fliC H21 might be repressed, H-phenotype is than determined by flmA H54.
fliC H23	fliC-H23_11	flagellin C H-antigen H23 (AY250005.1)
fliC H24	fliC-H24_11	flagellin C H-antigen H24 (AY250006.1)
fliC H25	fliC-H25_11	flagellin C H-antigen H25 (ADUP01000024.1)
fliC H26	fliC-H26_11	flagellin C H-antigen H26 (AY250008.1)
fliC H27	fliC-H27_11	flagellin C H-antigen H27 (CU928162.2)
fliC H28	fliC-H28_11	flagellin C H-antigen H28 (AY250010.1)
fliC H29	fliC-H29_11	flagellin C H-antigen H29 (AY250012.1)
fliC H30	fliC-H30_11	flagellin C H-antigen H30 (AY250011.1)
fliC H31	fliC-H31_11	flagellin C H-antigen H31 (AY250013.1)
fliC H32	fliC-H32_11	flagellin C H-antigen H32 (AY250014.1)
fliC H33	fliC-H33_11	flagellin C H-antigen H33 (AY250015.1)
fliC H34	fliC-H34_11	flagellin C H-antigen H34 (AY250016.1)
fliC H37	fliC-H37_11	flagellin C H-antigen H37 (AY250017.1)
fliC H38	fliC-H38_11	flagellin C H-antigen H38 (AY250018.1)
fliC H39	fliC-H39_11	flagellin C H-antigen H39 (AY250019.1)
fliC H40	fl-H40_11	flagellin C H-antigen H40 (AJ865464.1), Note: If repressor fljA "positive", fliC H40 might be repressed, H-phenotype is than determined by flkA H53.

fliC H41	fliC-H41_11	flagellin C H-antigen H41 (AY250020.1)
fliC H42	fliC-H42_11	flagellin C H-antigen H42 (AY250021.1)
fliC H43	fliC-H43_11	flagellin C H-antigen H43 (AY250022.1)
fliC H45	fliC-H45_11	flagellin C H-antigen H45 (AY250023.1)
fliC H46	fliC-H46_11,fliC-H46_12	flagellin C H-antigen H46 (AY250024.1)
fliC H48	fliC-H48_11	flagellin C H-antigen H48 (AY250025.1)
fliC H49	fliC-H49_11	flagellin C H-antigen H49 (AY250026.1)
fliC H51	fliC-H51_11	flagellin C H-antigen H51 (AY250027.1)
fliC H52	fliC-H52_11	flagellin C H-antigen H52 (AY250028.1)
flkA H53	flkA-H53_11	flagellin A H-antigen H53 (AB128917.1) (Note: If repressor FljA "positive", the gene fliC H40 is repressed; H-phenotype is H53)
flmA H54	flmA-H54_11	flagellin A H-antigen H54 (AB128918.1) (Note: If repressor FljA "positive", the gene fliC H21 is repressed; H-phenotype is H54)
fliC H56	fliC-H56_11	flagellin C H-antigen H56 (AY250029.1)
H-serotyping (additional marker)		
fliC Marker	fliC-5p_11, fliC-5p_12, fliC-5p_13, fliC-5p_14, fliC-5p_15	fliC marker - consensus sequence for all fliC genes
fljA Repressor	fljA_11	FljA represses the expression of fliC genes, if "positive" the genes flkA (H03 or H53) or flmA (H54) represent the H-serotype (AB128916.1)
fliC non-motile	fl-H-NM_11	flagellin C from non-motile isolates (AY337480.1)
streptogramin A resistance		
vatE	hp_vatE_611, hp_vatE_612	acetyltransferase;streptogramin A acetyltransferase; associated with resistance to streptogramin A (AF242872.1)
aminoglycoside resistance		
strA, strB	prob_strA_611, prob_strB_611	aminoglycoside-3''-phosphotransferase (locus A) and aminoglycoside-6''-phosphotransferase; associated with resistance to streptomycin (EF090911.1)
aac_aph	hp_aac_aph_611	3-N-aminoglycoside acetyltransferase; associated with resistance to gentamycin (AE017171.1)
aac3	hp_aac3_611, hp_aac3_612, hp_aac3_613, hp_aac3_614, hp_aac3_615	3-N-aminoglycoside acetyltransferase; associated with resistance to gentamycin (U90945.1)
aac3Ia	prob_aac3Ia_1	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin; sisomicin (U90945.1)
aac3IVa	prob_aac3IVa_1	3-N-aminoglycoside acetyltransferase; associated with resistance to apramycin; dibekacin; gentamicin; netilmicin; sisomicin; tobramycin (EU784152.1)
aac6	hp_aac6_611, hp_aac6_612, hp_aac6_613, hp_aac6_614, hp_aac6_616, hp_aac6_617, hp_aac6_618	aminoglycoside 6'-N-acetyltransferase, associated with resistance to amikacin; dibekacin; isepamicin; netilmicin; sisomicin; tobramycin (AF162771.1)

aac6Ib	prob_aac6Ib_1	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin (M21682.1)
aadA1	prob_aadA1_1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin (EU704128.1)
aadA2	prob_aadA2_1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin (EU704128.1)
aadA4	prob_aadA4_1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin (Z50802.3)
aadB	hp_aadB_611, hp_aadB-2_611	2''-aminoglycoside nucleotidyltransferase (L06418.4)
ant2	prob_ant2Ia_1	aminoglycoside (2'') adenylyltransferase; associated with resistance to dibekacin; gentamicin; kanamycin; sisomicin; tobramycin (L06418.4)
aphA	hp_aphA_611, hp_aphA-var4_611, hp_aphA-var5_611	aminoglycoside 3'-phosphotransferase; kanamycin resistance protein (AY260546.3)
grm	hp_grm_611	16S rRNA methylase, associated with gentamicin resistance (M55521.1)
armA	hp_armA_611	16S rRNA methylase, associated with aminoglycoside resistance (AB117519.1)
rmtA	hp_rmtA_611	16S rRNA methylase, associated with aminoglycoside resistance (AB083212.2)
rmtB	hp_rmtB_611	16S rRNA methylase, associated with aminoglycoside resistance (DQ345788.1)
rmtC	hp_rmtC_611	16S rRNA methylase, associated with aminoglycoside resistance (AB194779.2)
rmtD	hp_rmtD_611	16S rRNA methylase, associated with aminoglycoside resistance (DQ914960.2)
npmA	hp_npmA_611	16S rRNA methylase, associated with aminoglycoside resistance (AB261016.1)
beta lactam resistance		
blaACC	prob_acc2_11, prob_acc1_11	class C beta-lactamase blaACC-1/blaACC-2(EF554600.1)
blaACT	prob_act1_11	class C beta-lactamase blaACT-1(U58495.2)
blaCMY	hp_blaCMY_611, hp_blaCMY_612, prob_cmy_11	consensus sequence for blaCMY-13, blaCMY-2, blaCMY-24, blaCMY-35, blaCMY-CFE1, blaCMY-CFE2 (Citrobacter spp.), blaCMY-Cmur (Citrobacter murliniae), blaCMY-Cwer (Citrobacter werkmanii), blaCMY-Cyou (Citrobacter youngae), blaCMY-HG3, blaCMY-HG4
blaKHM	hp_blaKHM-1_611	class B metallo beta-lactamase (AB364006.1)
blaMOX-CMY9	hp_blaMOX-CMY9_611, hp_blaMOX-CMY9_612, hp_blaMOX-CMY9_613	class C extended-spectrum beta-lactamase precursor, associated with resistance to cephalosporins (AF381617.1)
blaCTX-M1, blaCTX-M15	prob_ctxM1_11, prob_ctxM1_12	class A extended-spectrum-beta-lactamase (X92506.1), including blaCTX-M15 (HQ202266.1)
blaCTX-M2, blaCTX-M8, blaCTX-M26	prob_ctxM2_11, prob_ctxM26_11, prob_ctxM8_11	class A extended-spectrum-beta-lactamase (AM040709.1, AF518567.2, AY750914.2)
ctxM9	prob_ctxM9_11, prob_ctxM9_12	class A beta-lactamase (AF174129.3)

blaDHA-1	prob_dha1_1	class C beta-lactamase (EF406115.1)
blaFOX	prob_fox_11, prob_mox_1mm	class C beta-lactamase blaFOX (consensus)
blaGES-1	hp_ges-1_611	class A beta-lactamase (AY219651.1)
blaGIM-1	hp_gim1_611	class B metallo beta-lactamase (AJ620678.1)
blaIMI-3	hp_imi3_611	class A metallo beta-lactamase - carbapenemase, associated with imipenem resistance (AY780889.1)
blaIMP	hp_imp_611, hp_imp_612, hp_imp_613, hp_imp_614, hp_imp_615, hp_imp_616, hp_imp_617	class B metallo beta-lactamase (consensus), associated with imipenem resistance
blaKPC-4	hp_kpc4_611	class A beta lactmase - carbapenemase (EU447304.1.)
blaLAP-1	hp_lap1_611	class A beta-lactamase (EF026092.1)
blaLEN-1	prob_len1_11	class A beta-lactamase (AY743416.1)
blaMOX	prob_mox_1pm	class C beta-lactamase blaMOX (consensus)
blaOXA-1	prob_oxa1_21	oxacillinase - class D beta-lactamase blaOXA-1 (AY458016.1)
blaOXA-2	prob_oxa2_11	oxacillinase - class D beta-lactamases blaOXA-2/blaOXA-15 (U63835.1)
blaOXA-7	prob_oxa7_11	oxacillinase - class D beta-lactamase blaOXA-7 (AY866525.1)
blaOXA-9	prob_oxa9_11	oxacillinase - class D beta-lactamase blaOXA-9 (M55547.1)
blaOXA-23	hp_oxa_611	oxacillinase - class D beta-lactamase (AJ132105.1)
blaOXA-40	hp_oxa_612	oxacillinase - class D beta-lactamase (AF509241.1)
blaOXA-48	hp_oxa_613	oxacillinase - class D carbapenem-hydrolyzing beta-lactamase (AY236073.2)
blaOXA-51	hp_oxa_614	oxacillinase - class D beta-lactamase (CP000863.1)
blaOXA-54	hp_oxa_615	oxacillinase - class D beta-lactamase (AY500137.1)
blaOXA-55	hp_oxa_616	oxacillinase - class D beta-lactamase (AY343493.1)
blaOXA-58	hp_oxa_617	oxacillinase - class D beta-lactamase (AY665723.1)
blaOXA-60	hp_oxa_618	oxacillinase - class D beta-lactamase (AF525303.2)
blaPER-1	hp_per1_611	class A beta-lactamase PER-1; extended-spectrum beta-lactamase (Z21957.1)
blaPER-2	hp_per2_611, prob_per2_1	class A beta-lactamase PER-2; extended-spectrum beta-lactamase (X93314.1)
blaPSE-1	prob_pse1_1pm, prob_pse1_1mm	carbenicillinase (Z18955.1)
blaSHF-1	hp_sfh1_611	class B metallo beta-lactamase (AF197943.1)
blaSHV	prob_shv1_11	class A beta-lactamase - consensus sequence for blaSHV genes, including extended-spectrum beta-lactamases
blaSME-1	hp_sme1_611	carbapenem hydrolysing beta-lactamase (Serratia marcescens)(Z28968.1)
blaSPM-1	hp_spm1_611	metallo beta-lactamase, carbapenemase (AY341249.1)
blaTEM	prob_tem1_1	class A beta-lactamase - consensus sequence for blaTEM genes, including extended-spectrum beta-lactamases

blaVEB-1	hp_veb1_611	extended-spectrum beta-lactamase (AF010416.1)
blaVIM	hp_vim_611, hp_vim_612, hp_vim_613	class B metallo beta-lactamase (Y18050.2)
ble	hp_ble_611	bleomycin resistance (X01702.1)
chloramphenicol resistance		
catA1	prob_catA1_11	chloramphenicol acetyltransferase (group A) (V00622.1)
catB3	prob_catB3_11	chloramphenicol acetyltransferase (group B) (AJ009818.1)
catB8	prob_catB8_12	chloramphenicol acetyltransferase (AF227506.1)
catIII	prob_catIII_1	chloramphenicol acetyltransferase (type III) (AJ249249.1)
cmlA1	prob_cmlA1_11	chloramphenicol transporter (EF113389.1)
floR	prob_floR_11	florfenicol export protein (AF252855.1)
erythromycin resistance		
ereA	hp_ereA_611, hp_ereA_612	type I erythromycin resistance (AY183453.1)
ereB	hp_ereB_611, hp_ereB_612	type II erythromycin resistance (AB207867.1)
macrolide resistance		
ermB	hp_ermB_611, hp_ermB_612	rRNA adenine N-6-methyltransferase, lincosamide and streptogramin B resistance protein (AB089505.1)
mphA	hp_mph2_611, hp_mphA_611, hp_mphB_611, hp_mphBM_611, hp_mphD_611,	macrolide 2'-phosphotransferase (EF102240.1)
mrx	hp_mrx_611	member of macrolide inactivation gene cluster mphA-mrx-mphR (AB038042.1)
quinolione resistance		
qepA	hp_qepA_611	QepA - fluoroquinolone/quinolone efflux pump (AM886293.1)
qnrA1	prob_qnr_11, prob_qnr_12	quinolone or fluoroquinolone resistance protein (AY931018.1)
qnrB	prob_qnrB_11, prob_qnrB_12	quinolone or fluoroquinolone resistance protein (AB281054.1)
qnrD	hp_qnrD_611	quinolone or fluoroquinolone resistance protein (FJ228229.1)
qnrS	prob_qnrS_11	quinolone or fluoroquinolone resistance protein (AM234722.1)
rifampin resistance		
arr	hp_arr-1_611, hp_arr-4_611, hp_arr-5_611, hp_arr-6_611	ADP-ribosyltransferase, associated with resistance to rifampin (AF078527.1)
streptomycin resistance		
sph	hp_sph_611	streptomycin 3''-phosphotransferase (U00004.1)
streptomycin resistance		
sul1	prob_sul1_11	dihydropteroate synthetase type 1 (AJ698325.1)
sul2	prob_sul2_11	dihydropteroate synthetase type 2 (DQ464881.1)
sul3	prob_sul3_11	dihydropteroate synthetase type 3 (AJ459418.2)

tetracycline resistance		
tet37	hp_tet37_611	tetracycline resistance protein (AF540889.1)
tetA	prob_tetA_11	tetracycline resistance protein A, tetracycline efflux protein (CP000971.1)
tetB	prob_tetB_11	tetracycline resistance protein A, class B (V00611.1)
tetC	prob_tetC_11	tetracycline resistance protein A, class C (EU751612.1)
tetD	prob_tetD_1	tetracycline resistance protein A, class D (X65876.1)
tetE	prob_tetE_11	tetracycline resistance protein A, class E (L06940.1)
tetG	prob_tetG_11, prob_tetG_12	tetracycline resistance protein A, class G (AF261825.2)
tetX	hp_tetX_611	tetracycline resistance protein (M37699.1)
trimethoprim resistance		
dfrA1	prob_dfrA1_21, prob_dfrA1_22	dihydrofolate reductase type 1 (AJ884723.1)
dfrA5	prob_dfrV_21	dihydrofolate reductase type 5 (AB188269.1)
dfrA7	prob_dfrA7_11, prob_dfrA7_12	dihydrofolate reductase type 7 (AB161450.1, AM237806.1)
dfrA12	prob_dfr12_11	dihydrofolate reductase type 12 (AB154407.1)
dfrA13	prob_dfr13_11	dihydrofolate reductase type 13 (synonym A21) (Z50802.3)
dfrA14	prob_dfrA14_21	dihydrofolate reductase type 14 (AJ313522.1)
dfrA15	prob_dfrA15_1	dihydrofolate reductase type 15 (Z83311.1)
dfrA17	prob_dfrA17_11	dihydrofolate reductase type 17 (AF169041.1)
dfrA19	prob_dfrA19_1	dihydrofolate reductase type 19 (AJ310778.1)
virulence factor - adhesins		
eae - consensus	eae_consensus_10, eae_consensus_20, eae_consensus_30, eae_consensus_40	an outer membrane protein important for the attachment to host cells; pathogenesis factor (M58154.1)
efa1	hp_efa1_611	lymphocyte inhibitory factor A - adherence factor (AF159462.2)
fasA	fasA_10	adhesin - fimbrial major subunit (M35257.1)
fedA	fedA_10	adhesin - fimbrial major subunit (M61713.1)
fedF	fedF_10	adhesin - fimbrial protein (Z26520.1)
fim41a	fim41a_10	adhesin - fimbrial protein (X14354.1)
iha	hp_iha_611	adherence-conferring protein (BA000007.2)
nfaE	nfaE_10	chaperone protein - required for the expression of aggregative adherence fimbria II (S61968.1)
espB_O157	espB_O157_20	EspB - protein (type III secretion system; O157:H7) (BA000007.2)
espB_O26	espB_O26_40	EspB - protein (type III secretion system, 26:H- and O15:H-) (AJ287768.1)
saa	hp_saa_611	STEC autoagglutinating adhesin (AF399919.3)

virulence factor - fimbrae		
bfpA	bfpA_10	BfpA protein - essential for apoptosis signalling (AB024946.1)
cfaC	cfa_c_10	outer membrane usher protein (M55661.1)
cofA	cofA_10	major pilin subunit - CFA/III pilin (D37957.1)
f17-A	f17-A_40, f17-A_50, f17-A_60	major fimbrial subunit protein (L77091.1)
f17-G	f17-G_20	major fimbrial subunit protein (pilin G) (L43372.1)
fanA	fanA_10	regulatory protein (X05797.1)
K88ab	K88ab_10	major subunit of K88 fimbriae (V00292.1)
lngA	lngA_20	longus pilus structural subunit (EF595770.1)
lpfA	hp_lpfA_611	major fimbrial subunit (AY057066.1)
perA	perA_10, perA_20	transcriptional activator (AF255772.1)
prfB	prfB_30	major pilu subunit operon regulatory protein (X76613.1)
sfaS	sfaS_10	adhesin - minor Shigella fimbriae subunit (X16664.4)
virulence factor - secretion systems		
cif	hp_cif_611	cell cycle inhibiting factor (type III secretion system) (AY128535.1)
espA_C_rodentium	hp_espA_Crod_611	EspA - protein (type III secretion system), associated with Citrobacter rodentium (AF311901.1)
espA	hp_espA_O103H2_611, hp_espA_O119H6_611, hp_espA_O127H7_611, hp_espA_O157H11_611, hp_espA_O49H12_611, hp_espA_O55H7_611, hp_espA_O8_611	EspA - protein (type III secretion system) (AF054421.1)
espC	hp_espC_611	EspC - extracellular serine protease (type III secretion system) (AF297061.1)
espF	hp_espF_611, hp_espF_612	EspF - effector protein (type III secretion system) (AE005174.2)
espF_C_rodentium	hp_espF_Crod_611	EspF - effector protein (type III secretion system) (AF311901.1)
espF_O103H2	hp_espF_O103H2_611, hp_espF_O103H2_612	EspF - effector protein (type III secretion system) (AJ277443.1)
espI	hp_espI_611	EspI - non-LEE encoded effector protein (LEE - EPEC Locus of Enterocyte Effacement) (AJ278144.1)
espJ	hp_espJ_611, hp_espJ_612	EspJ - non-LEE encoded effector protein (LEE - EPEC Locus of Enterocyte Effacement) (AE005174.2)
etpD	hp_etpD_611	EtpD - type II secretion pathway related protein (AF074613.1)
nleA	hp_nleA_611, hp_nleA_612, hp_nleA_613, hp_nleA_614	NleA - non-LEE-encoded effector protein A (type III secretion system) (AM421997.1)
nleB	hp_nleB_611	NleB - non-LEE-encoded effector protein B (type III secretion system (BA000007.2)
nleB O157:H7	hp_nleB_O157H7_611	NleB - non-LEE-encoded effector protein B (type III secretion system), associated with serotype O157:H7 (BA000007.2)

nleB Salmonella	hp_nleB_Styp_611	NleB - non-LEE-encoded effector protein B (type III secretion system), associated with Salmonella enterica (AE008894.1)
nleC	hp_nleC_611	NleC - non-LEE-encoded effector protein C (type III secretion system) (AY485823.1)
tccP	hp_tccP_611, hp_tccP_612	Tir - cytoskeleton coupling protein (type III secretion system) (AB275113.1)
virulence factor - SPATE (serin protease autotransporters)		
espP	hp_espP_611	EspP - serine protease autotransporter of Enterobacteriaceae (SPATE) (AF074613.1)
pic	hp_pic_611	Pic - serin protease autotransporter of Enterobacteriaceae (SPATE) (U35656.1)
rpeA	hp_rpeA_611	RpeA - serin protease autotransporter of Enterobacteriaceae (SPATE) (AY552473.1)
sepA	hp_sepA_611	SepA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AY604009.1)
sigA	hp_sigA_611	SigA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AF200692.2)
tsh	hp_tsh_611	Tsh - hemoglobin-binding protease (SPATE) (AJ223631.1)
vat	hp_vat_611	Vat - haemoglobin protease (SPATE) (AF242872.1)
eaaA	hp_eaaA_611	EaaA - serine protease autotransporter of Enterobacteriaceae (SPATE)(AF151674.1)
eatA	hp_eatA_611	EatA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AY163491.2)
epeA	hp_epeA_611	EpeA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AY258503.2)
astA	astA_consens_10	heat stable enterotoxin (consensus sequence)
virulence factor - SPATE (serin protease autotransporters)		
cba	cba_10	colicin B activity protein (M16816.1)
ccl	ccl_10	colicin activity protein (AF540491.1)
cdtB	cdtB_40, cdtB_50, cdtB_60	cytolethal distending toxin subunit B (AJ508930.1)
celB	celb_10	colicin lysis protein (X03632.1)
cma	cma_20	colicin M activity protein (CP000971.1)
cnf1	cnf1_20	cytotoxic necrotizing factor type 1 (CP000243.1)
hlyA	hlyA_20	hemolysin A (AB011549.2)
hlyE	hlyE_10	hemolysin E (AF052225.1)
ipaD	ipaD_10	IpaD - invasin (CP000035.1)
ipaH	ipaH9.8_20	IpaH - invasion plasmid antigen (CP000039.1)
ltcA	ltcA_20	heat labile enterotoxin subunit A (AB011677.1)
mchB	mchB_10	microcin H47 activity protein (AJ515252.1)
mchC	mchC_20	member of the microcin operon (AJ515252.1)
mchF	mchF_10	putative microcin L transport protein (AJ515252.1)
mcmA	mcmA_10	microcin M truncated protein (AJ515252.1)
pet	pet_20	enterotoxin (SPATE) (AF056581.1)
sat	hp_sat_611	Sat serine protease (SPATE) (AJ586888.1)

senB	senB_20	enterotoxin (Z54195.1)
sta1	sta1_110	heat stable enterotoxin I (AJ555214.1)
sta2	sta2_210	heat stable enterotoxin II (CP000795.1)
stb	stb_10	heat stable enterotoxin Stb - enterotoxin B (M35729.1)
stx1 - consensus	stx1A_10	shiga toxin 1
stx2 - consensus	hp_stxA2_611, hp_stxA2_613, hp_stxA2_614, hp_stxA2_615, hp_stxA2_616, hp_stxA2_617, hp_stxA2_618, stx2A_10, hp_stxB2_612, hp_stxB2_613, hp_stxB2_614, hp_stxB2_615	shiga toxin 2 (NOTE: variant classification by Scheutz et al. 2012)
stx2b	hp_stxB2_612	shiga toxin 2 variant b (AB012101.1)
stx2e	hp_stxA2_616	shiga toxin 2 variant e (X81415.1)
stx2f	hp_stxA2_611, hp_stxA2_613	shiga toxin 2 variant f (AJ270998.1)
stx2g	hp_stxA2_617	shiga toxin 2 variant g (AJ966782.1)
stx2a,c,d	hp_stxA2_614, hp_stxA2_618, hp_stxB2_614, stx2A_10	shiga toxin 2 variant a, shiga toxin 2 variant c or shiga toxin 2 variant d (X61283.1)
subA	hp_subA_611	subtilase cytotoxin, subunit A (AF399919.3)
toxB	hp_toxB_611, hp_toxB_612, hp_toxB_613	cytotoxin B (AB011549.2)
virF	virF_20	transcriptional regulator - required for transcription of virB and icsA (AF348706.1)
virulence factor - miscellaneous		
hemL	hp_hemL_612	glutamate-1-semialdehyde aminotransferase (U00096.2)
intI1	prob_intI1_1	class 1 integron integrase (AY260546.3)
intI2	prob_intI2_11	class 2 integron integrase (AY183453.1)
ireA	ireA_20	siderophore receptor - iron-regulated outer membrane protein (AF320691.1)
iroN	iroN_10	outer membrane siderophore receptor (AF449498.2)
iss	iss_10	increased serum survival (AF042279.1)
katP	hp_katP_611	peroxidase and catalase (AB011549.2)
tir	hp_tir_4051.6_611, hp_tir_MPEC_611, hp_tir_O103H2_611, hp_tir_O111_611, hp_tir_O157H45_611, hp_tir_O157H7_611, hp_tir_NTH19_611	translocated intimin receptor (consensus)

APPENDIX 3 – TYPING INFORMATION

Definitions & Explanations

The displayed result will yield following typing information:

- Discrimination of the 23 described O-serotypes is mainly determined by the genes *wzy* (polymerase) and *wzx* (flippase). The 47 known H-antigens are encoded by the gene *fliC*.
- The probes immobilized on the current array version can discriminate 23 O-antigens:

  O:4, O:6, O:7, O:8, O:9, O:15, O:26, O:52, O:53, O:55, O:79, O:86, O:91, O:101, O:103, O:104, O:111, O:113, O:114, O:121, O:128, O:157 and O:172
- The following flagellar antigens can be identified on the array:

  H:01, H:02, H:03, H:04, H:05, H:06, H:07, H:08, H:09, H:10, H:11, H:12, H:14, H:15, H:16, H:18, H:19, H:20, H:21, H:23, H:24, H:25, H:26, H:27, H:28, H:29, H:30, H:31, H:32, H:33, H:34, H:37, H:38, H:39, H:40, H:41, H:42, H:43, H:45, H:46, H:46, H:48, H:49, H:51, H:52, H:53, H:54, H:56
- The mix culture control is based on 5 probes located in consensus sequences on the 5' end of the *fliC* genes. These probes were divided in two groups which are correlated with two groups of *fliC* genes (fliC-5p_11, fliC-5p_12 and fliC-5p_13, fliC-5p_14, fliC-5p_15). Only one group should be positive, if not an *E. coli* mix culture was probed.
- The gene *fljA* encodes for a repressor protein which repressed some of *fliC* genes. If the *fljA* probe positive may two H- serotype probes are positive. In this case the genes *flkA* and *flmA* encodes for the H-serotype. Such strains are described as biphasic (Wang *et al.* 2003, Tominaga 2004).
- Probes specifying *gapA, gad*, *ihfA,* and *dnaE* that were introduced to confirm the identity of *E. coli* and to serve as genus controls.
- Probes used to detect the following antimicrobial resistance and virulence genes: *see Appendix 2 -Probe to Target Table*